Urol Res (2002) 30: 324–328
DOI 10.1007/s00240-002-0278-x

ORIGINAL PAPER

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Contralateral genitofemoral sympathetic nerve discharge increases following ipsilateral testicular torsion

Received: 23 March 2002 / Accepted: 29 July 2002 / Published online: 7 September 2002

Abstract The decrease in blood flow due to the activation of sympathetic system has been suggested to play a role in contralateral testicular deterioration associated with unilateral testicular torsion. Sympathetic nerve discharges (SND) from the genitofemoral nerve were evaluated before and during unilateral testicular torsion. Under urethane anesthesia, arterial blood pressure and SND from splanchnic and right genitofemoral nerves were recorded in 12 male Sprague-Dawley rats, 8 of which were included in subsequent analyses. After control recordings of basal discharges for 2 min the left testis was twisted 720° counterclockwise, and recording was resumed for an additional 30 min. Changes in nerve activity were calculated by measuring the area under the autospectrum curve, and alterations were compared. Following testicular torsion no significant changes were obtained for splanchnic SND, but the amplitude of SND from contralateral genitofemoral nerve showed an overall increase of $21.20 \pm 7.03\%$ in six rats. This increase lasted about 10–15 min and activities returned to pretorsion levels. In two other rats no significant change was observed in either splanchnic or genitofemoral SND. Ipsilateral testicular torsion results in a transient increase in genitofemoral SND. A possible autonomic reflex mechanism may exist, and it may be activated by noxious stimuli from contralateral side. This reflex mechanism may initiate a series of events that lead to the injury of contralateral testis.

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Keywords Spermatic cord torsion · Testis · Contralateral testicular injury · Infertility · Sympathetic outflow

Introduction

Both experimental and clinical studies have shown that unilateral testicular torsion results in damage not only to the involved but also to the contralateral, uninvolved testis [2, 15]. Although various mechanisms such as autoimmunity, underlying congenital defect, and subclinical attacks of contralateral testicular torsion have been proposed to explain the contralateral testicular damage, none has yet received universal acceptance [23]. Previous studies have showed that following testis torsion blood flow decreases [18, 23], and testicular arterioles collapse [6] on the contralateral side. In addition, there is an increase in the syntheses of biochemical indicators of tissue hypoxia [1, 3] and a decrease in tissue oxygen content [18] and adenylate energy charge [5]. Tissue noradrenaline content also decreases [24]. These results suggest the involvement of the sympathetic nervous system in the development of damage to the contralateral testis. Another line of evidence supporting the involvement of the sympathetic nervous system came from the studies in which chemical sympathectomy [11, 12, 17] or prior afferent nerve denervation [19, 20] prevents the damage to the contralateral testis. These studies have led us to propose an alternative hypothesis based on the involvement of sympathetic nervous system [23]. According to this hypothesis, SND to contralateral testis increases following the torsion procedure and results in testicular damage by vasoconstriction of the arterioles feeding the contralateral testis.

However, changes in discharges of sympathetic nerves innervating the genital organs, either intact or during testicular torsion, have not been previously studied. This experimental study monitored the changes in genitofemoral SND which contains sympathetic fibers projecting to the testis. We hypothesized that SND to

the contralateral testis increases following unilateral testicular torsion provided that an autonomic reflex mechanism exists.

Materials and methods

General procedures

All protocols used in this study on male Sprague-Dawley rats (280–320 g) were approved by the Research Animals Ethics Board of Hacettepe University (DHEK 01-36-2). Rats were anesthetized with an intraperitoneal injection of urethane (1.1 g/kg), paralyzed (gallamine triethiodide, 4 mg/kg intravenously, initial dose), pneumothoracotomyzed, and artificially respirated with 100% O_2. End-tidal CO_2 was held near 3–4% (CWE Instruments Capstar 100, Ardmore, Pa.,USA), and rectal temperature was kept near 37°C with a hot water circulating blanket. Before neuromuscular blockade the adequacy of anesthesia was assessed by the absence of responses to noxious stimuli (e.g., pinch, heat, surgery). The right femoral artery and both femoral veins were cannulated to record blood pressure and to administer drugs.

Baroreceptor nerves were kept intact in all animals, and mean arterial pressure (MAP) was maintained at the same level throughout the analysis period (i.e., before and after testicular torsion). To avoid blood pressure related changes in SND which might occur as the result of a change in the level of baroreceptor nerve activity an intravenous infusion (Harvard Apparatus Model 600–000, Holliston, Mass., USA) of a mixture of phenylephrine HCl (50 μg/ml) and rheomacrodex (6% in saline) was used to set MAP at a steady level (near 100 mmHg). The rate of infusion was adjusted during the experiment to keep MAP constant.

Testicular torsion was performed on the testis contralateral to the recording side. Through a scrotal incision the left testis was carefully twisted for 720° in counterclockwise direction.

Neural recordings and experimental procedure

Rats were mounted on a stereotaxic frame and spinal unit (David Kopf Instruments, Tujunga, Calif., USA). The right genitofemoral and splanchnic nerves were isolated retroperitoneally. Nerves were isolated and cut, and the central ends were mounted on platinum electrodes. Retroperitoneal cavity was filled with mineral oil to assure electrical isolation. Monophasic recordings of postganglionic SND were made with a preamplifier band pass of 1–1000 Hz. The synchronized discharges of populations of sympathetic nerve fibers appear as slow waves (i.e., envelopes of spikes) when this band pass is used [8, 14].

At the beginning of the experiments a bolus injection of phenylephrine (25 μg/kg, 0.1 ml) was made to activate the baroreceptor reflex (i.e., baroreflex testing). Inhibition of SND following the abrupt increase in arterial pressure was taken as an indicator of sympathetic nature of the nerve recordings. This test was deemed necessary as experiments with genitofemoral nerve recording is limited in the literature. When all variables returned to their prebaroreflex test levels, simultaneous recordings of arterial pressure and genitofemoral and splanchnic nerve activities for 2 min were obtained. These recordings served as control before torsion. After torsioning the testis arterial pressure and genitofemoral and splanchnic nerve activities were recorded for 30 min. Prior to all the analyses rats that showed fluctuations in physiological parameters ($n=4$) which may affect SND such as changes in blood pressure were discarded from the study.

Data analysis

SND was low-pass filtered at 100 Hz before all analyses were made on a Macintosh PowerBook G3 computer. Data were acquired (400 Hz sampling rate) with software (Chart, version 3.6) and a data acquisition system from ADInstruments (MacLabs series, Castle Hill, Australia). Frequency-domain analysis was made using the built-in module of the Chart software. Fast Fourier transform was performed on 48 contiguous windows of 1024 data points with no overlap (122.88 s data block) to construct autospectra of genitofemoral and splanchnic SND. Spectral analyses were carried out over a frequency band of 0–200 Hz with a resolution of 0.4 Hz/bin. The figures in this report show only frequencies higher than 20 Hz because at least 90% of the total power in SND was found within this band [10]. The autospectrum of a signal shows how much power (voltage squared) is present at each frequency. The autospectra of SND before and after testicular torsion were displayed on the same power scale. Total SND power was calculated by arithmetically summing the values for the bins in 0–20 Hz frequency range. Total power was defined as the sum of the values for the bins between 0 and 20 Hz.

Statistical analysis

Data were expressed as means ±SE. Student's t test for one group (StatView 4.5, Abacus Concepts) was used to compare MAP and power in SND before and after testicular torsion. Raw values of power were used for statistical analyses, but changes in SND were expressed as percentage of control in the text. P values less than 0.05 were taken as indicating statistical significance.

Results

Data analysis was performed on eight rats in which simultaneous recordings of SND was made from genitofemoral and splanchnic nerves. An abrupt increase in blood pressure by bolus phenylephrine injection (i.e., baroreflex activation) temporarily abolished nervous activity in both nerves (Fig. 1). This was taken as an indication of sympathetic nature of the recorded nerve activity. Figure 2 illustrates an example of genitofemoral and splanchnic SND before and after testicular torsion. Genitofemoral SND was found increased following torsion of the contralateral testis. However, this increase

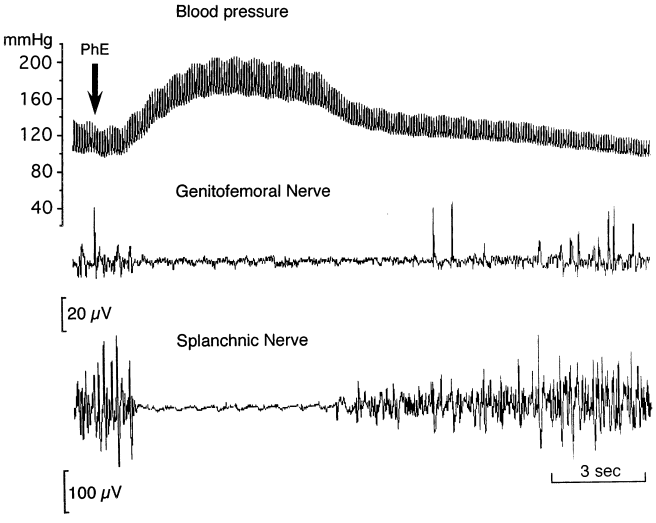

Fig. 1. The effects of baroreceptor reflex activation by an intravenous bolus phenylephrine (*PhE*, 25 μg/kg) injection on arterial blood pressure and on genitofemoral and splanchnic SND. *Arrow* Injection time

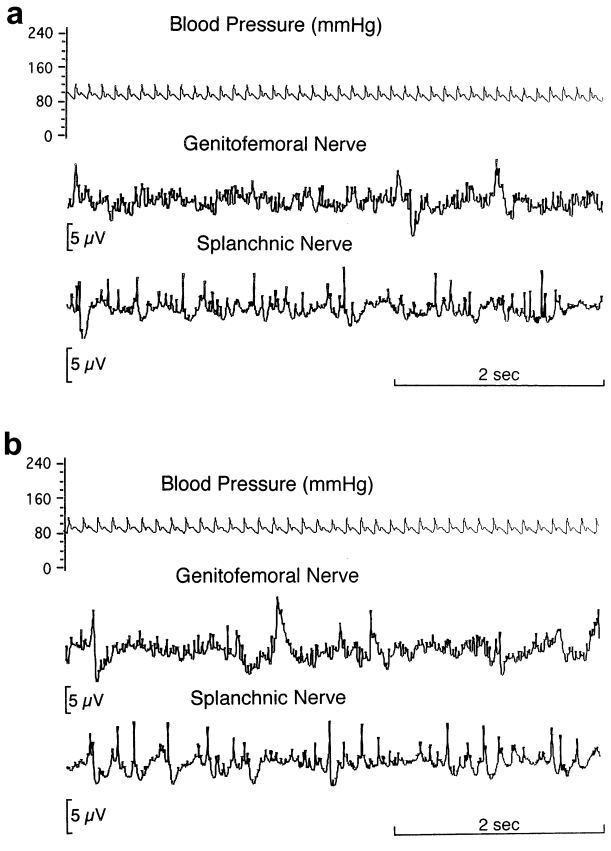

Fig. 2a, b. Effects of unilateral testicular torsion on SND and blood pressure. **a** Steady-state blood pressure and nerve activities, serving as control before torsion. **b** Blood pressure and SND 2 min after torsioning left testis 720°

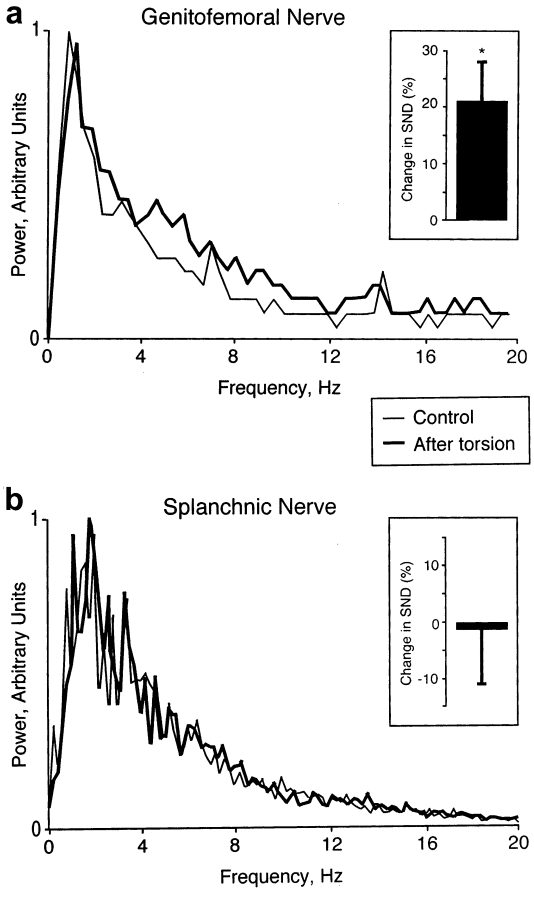

Fig. 3a, b. Effects of unilateral testicular torsion on genitofemoral and splanchnic SND. **a** Superimposed autospectra obtained from genitofemoral SND before and after (*bold lines*) testicular torsion. **b** The same for splanchnic SND. SND data for both nerves were obtained from simultaneous recordings. *Insets* Mean ± SEM values for six animals. $*P < 0.05$

was not sustainable as the activity returned to its pre-torsion levels within 10–15 min.

Spectral analysis was performed to quantify the changes in SND. In both nerves the majority of activity was found between 0 and 20 Hz. In animals in which baroreceptor entrainment was strong (i.e., one to one locking of sympathetic activity with the arterial pulse) a sharp peak at the cardiac frequency was detected. In three animals cardiac peak was either small or buried in the background nerve activity; however, baroreceptor reflex testing abolished the SND in those animals. Therefore all animals were included in the study irrespective of the presence of cardiac peak. MAP was measured at 96.7 ± 7.0 before and 97.9 ± 7.2 mmHg after torsion ($P > 0.05$). End-tidal CO_2 levels were between 3.2% and 3.8% in those experiments. Figure 3 shows the superimposed autospectra obtained from nerve recordings before and after testicular torsion. In genitofemoral SND a slight but statistically significant ($P < 0.05$) increase was obtained in six of eight animals. The increase in SND was uniform throughout the frequency spectrum and corresponds to an overall increase of 21.20 ± 7.03% (Fig. 3a, inset). Whereas splanchnic SND revealed a 1.36 ± 9.97% decrease after torsion (Fig. 3b, inset). This decrease was not statistically significant.

In the remaining two rats, testicular torsion failed to induce any changes in both genitofemoral and splanchnic SND.

Discussion

This study is the first to show that in terms of sympathetic discharges the genitofemoral nerve has similar characteristics as the splanchnic nerve. The splanchnic nerve served as control to assess the activity of the genitofemoral nerve; our findings of steady-state splanchnic SND are in line with those of other studies carried out in rats [13] and in cats [4]. Spectral analysis also permits qualitative assessment of the nerve activity over a frequency spectrum. Genitofemoral and splanchnic nerves displayed similar spectral features. First, the majority of the activity was between 0 and 20 Hz; second, a peak at cardiac frequency was present in the majority of the experiments; and, third, a broad band activity at 2–6 Hz was present in rats with poor baroreceptor afferent input. In addition, baroreceptor reflex activation completely abolished the genitofemoral SND.

We kept the blood pressure and end-tidal CO_2 levels constant to prevent possible fluctuations that could interfere with the level of baroreceptor afferent activity. Since we were not interested particularly in the study of cardiovascular parameters, we included all animals regardless of whether they had a prominent cardiac peak. Testicular torsion produced a modest but statistically significant increase in genitofemoral SND in the majority of rats without any significant change in splanchnic SND. There are at least three possibilities to explain why the increase in genitofemoral SND was limited. First, anesthesia may attenuate autonomic reflex responses [22]; second, genitofemoral nerve is a mixed nerve carrying both motor, and sensory fibers [16]; third, only a subset of genitofemoral nerve fibers reaches the testis [16]. In addition, absence of increase in genitofemoral SND in two rats which underwent testicular torsion may reflect possible variations among individuals.

The increase in genitofemoral SND was short lived (i.e., activity returned to baseline level within 10–15 min). Testis torsion may be considered a noxious stimulus, and the information is carried to the central nervous system by sensory afferents. As a whole, the results of this study are suggestive of a reflex mechanism that involves an increase in SND in the contralateral side. Previous studies have shown that administration of noradrenaline via testicular artery almost stops the blood flow to the testis [7, 21]. It can be postulated that sympathetic activation results in vasospasm in testicular tissue leading to a decrease in blood flow. Reflex increase in SND may offer an explanation for previously reported decreases in blood flow and oxygen content [18] as well as increases in biochemical indicators of tissue hypoxia [1, 3] and consumption of noradrenaline following testicular torsion [24]. It should be noted that all these effects can be prevented by prior chemical sympathectomy [11, 12, 17] or afferent nerve destruction [19, 20]. However, in a previous study we showed that the blood flow to the contralateral testis remained decreased for longer (i.e., hours) periods [18, 23]. This may indicate that such a putative mechanism plays an initiating role in the development of contralateral testicular injury as SND activity increase lasts only minutes following the torsion of the testis.

The increase in genitofemoral SND was specific to that nerve. Unlike the baroreceptor reflex, where the activity of all sympathetic nerves were affected indiscriminately, following testicular torsion, only genitofemoral SND was found increased. This finding may have important implications. Recent studies on so-called "fight or flight" responses show that sympathetic outflow may be affected differentially, producing opposite actions (i.e., increase in muscular blood flow, decrease in visceral blood flow) on different vascular beds [9]. Nevertheless, it is not clear whether this selective action on genitofemoral SND involves higher brain centers such as the medulla oblongata and hypothalamus and deserves further investigation.

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